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Stability of the Key Substances in *Isaria tenuipes* Extracts in Cosmetic Products

Jiraporn Pimthong[1], Aree Rittiboon[2], Tuntikorn Termkaew[2], Pimsumon Jiamboonsri[3], Marisa Jatupornpiput[2*]

[1] Thailand Institute of Nuclear Technology (Public Organization), Nakhon Nayok, Thailand

[2] Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand

[3] Faculty of Medical, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand

***Corresponding author e-mail: marisa.ja@kmitl.ac.th**

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Abstract

This paper quantified key bioactive substances in *Isaria tenuipes* extracts on a dry weight basis, including cordycepin (0.32 mg/g), adenosine (5.62 mg/g), and polysaccharides (65.8 mg/g). Antioxidant activity was evaluated using DPPH and ABTS assays, yielding IC_{50} values of 0.27 mg/g and 0.04 mg/g, respectively. Stability testing demonstrated that polysaccharides and adenosine remained relatively stable (less than 10% degradation) after 12 weeks of storage at both 4°C and 30°C, whereas cordycepin exhibited a greater reduction. The optimal formulation for incorporating *I. tenuipes* extract into a serum product was identified as 2.0% (w/w). After 12 weeks of storage at 30°C, the primary active compounds in the serum showed minimal degradation (<10%), while the pH, viscosity, and color remained stable at 4°C. Microbiological analysis confirmed compliance with industrial safety standards. Consumer evaluation using a 5-point hedonic scale (n = 100) indicated high satisfaction with moisturization (4.02), texture (3.93), absorption (4.10), and overall preference (4.06), outperforming a commercial reference product. These findings highlight the stability and cosmetic potential of *I. tenuipes* extract-based formulations.

Keywords: Adenosine, Cordycepin, *Isaria tenuipes*, Polysaccharide, Stability

1. Introduction

Isaria tenuipes, formerly known as *Paecilomyces tenuipes*, (Figure 1A.), is a parasitic fungus commonly found in mountainous areas in Korea (Kang et al., 2010). It typically grows on caterpillars and is recognized for its high nutritional value as well as its medicinal properties (Sapkota et al., 2011). *I. tenuipes* has been found to contain many important and active substances, *e.g.* (Wang et al., 2022; Wu et al., 2016) superoxide dismutase (Sharma, 2015), adenosine and cordycepin or 3'-deoxyadenosine (Du, 2012; Wu et al., 2016). These substances are known for their beneficial effects and have been incorporated into cosmetic products such as facial serums, enhancing the value of *I. tenuipes* in the cosmetic industry. However, to ensure product efficacy and quality, the concentrations of these key compounds must be controlled to meet minimum effective levels, and their stability during storage and use must also be maintained.

Globally, cosmetic use is increasing among both teenagers and adults, with consumers increasingly favoring products made from natural and organic ingredients due to their perceived ecological friendliness, safety, and health benefits (Antignac et al., 2016; Wu et al., 2016). However, botanical and fungal extracts often face limitations in terms of stability. *I. tenuipes* presents a promising natural ingredient for cosmetics, offering multifunctional bioactivities, particularly anti-aging, moisturizing, anti-inflammatory, and antimicrobial effects. With rising interest in fungal-derived compounds, this species has the potential to become a key component in next-generation skincare formulations.

In this study, we focused on the characterization and stability of key bioactive compounds in *I. tenuipes* extracts, including polysaccharides, cordycepin, adenosine, and other antioxidant substances. Serum formulations

incorporating the extracts were also analyzed to evaluate the stability of these active ingredients and to ensure their effectiveness in cosmetic applications, such as skincare serums. Furthermore, the physical, chemical, and microbiological properties were assessed to verify compliance with industrial product standards.

2. Materials and Methods

2.1 Materials

The materials were used for preparation base serum (Table 1).

Table 1. Materials were used for preparation base serum.

Material	Source	Notes
disodium EDTA	Namsiang (NSG) Co., Ltd.	Cosmetics grade
simulgel EG	The Real Three (Thailand) Co.,Ltd.	Cosmetics grade
cremophor RH40	Namsiang (NSG) Co., Ltd.	Cosmetics grade
phenostat	Namsiang (NSG) Co., Ltd.	Cosmetics grade
sorbitol	Namsiang (NSG) Co., Ltd.	Cosmetics grade
glycerin	Namsiang (NSG) Co., Ltd.	Cosmetics grade
propylene glycol	Namsiang (NSG) Co., Ltd.	Cosmetics grade
pro Polymer	Chanjao Longevity Co., Ltd.	Cosmetics grade

2.2 Extraction from *Isaria tenuipes*

Cordycepin, adenosine, and polysaccharides were extracted by adding 5 g of *I. tenuipes* powder to 100 ml distilled water, followed by heating in a sonicator bath at 40°C for 2 hours, as described by Choi et al. (2017). The extract was then freeze-dried, and the resulting powder was stored in a desiccator until further use.

2.3 Analysis of key substances and bioactive substances

Water-soluble extracts from *I. tenuipes* (Figure 1B.) were analyzed for cordycepin and adenosine by High-Performance Liquid Chromatography (HPLC) (Li et al., 2015). The chromatographic conditions were as follows: C18 column (4.6 × 150 mm, 5 µm), mobile phase consisting of water:methanol (85:15, v/v), flow rate of 1.0 mL/min, injection volume of 10 µL, and detection wavelength at 260 nm. Polysaccharide content was determined by the Anthrone assay (Kanlayavattanakul & Lourith, 2012). Antioxidant activity of the extracts, representing the presence of free radical-scavenging substances, was evaluated using the DPPH and ABTS assays (Dreywood, 1946; Kanlayavattanakul & Lourith, 2012).

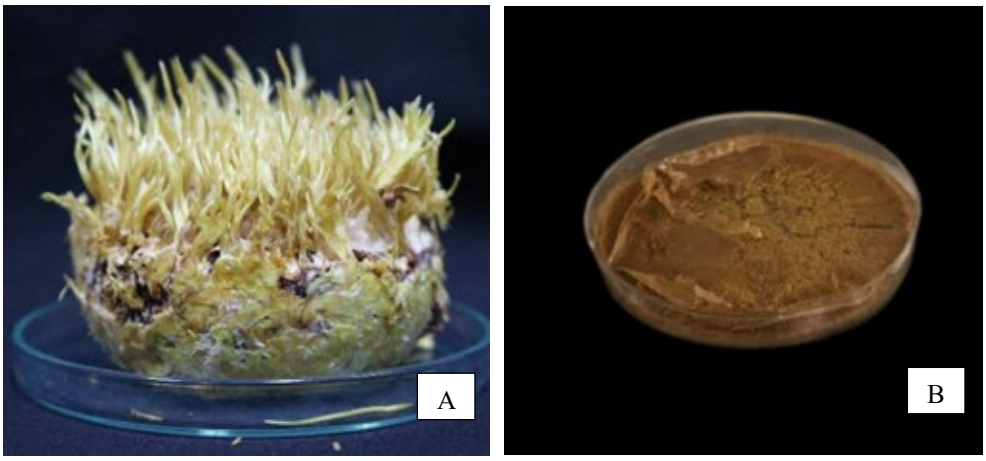

Figure 1. The characteristics of *I. tenuipes* (A) and *I. tenuipes* extract from freeze drying (B).

2.4 Stability test

Water-soluble extracts of *I. tenuipes* were stored at 4°C and 30°C. The stability of key substances, including cordycepin, adenosine, and polysaccharides, was analyzed every three weeks over a three-months period.

2.5 Test satisfaction of the basic serum formula

We prepared two serum formula recipes (Pakpiangchan & Chusuwan, 2018), then assessed the quality and satisfaction with 36 volunteers by selecting volunteers with good skin health (according to the product industry standards TISI Certification 478-2555) to select a good basic serum recipe.

Formula 1: 60 mg disodium EDTA (Munisekhar, 2014) was dissolved in 20 g water until homogeneous, then we added 2 g sorbitol, 2 g propylene glycol, and 0.5 g glycerin, followed by 3.5 g Simulgel EG which was stirred until a gel was created, gradually add water to the specified volume. Then add 0.2 g Cremophor RH40 and 1 g Phenostat. Stir to dissolve into a homogeneous mixture.

Formula 2: Use 60 mg disodium EDTA, dissolved in 20 ml water until homogeneous, then add 2 g sorbitol, 2 g propylene glycol, 0.5 g glycerin, and dissolve 0.8 g Pro Polymer in 20 g of water. Stir until the gel was created, then slowly add water to the specified volume, then add 0.2 g Cremophor RH40 and 1 g of Phenostat to and stir until dissolved.

Assessing physical properties observe creaming, cracking, viscosity, and color measured in the L*a*b* color space. Check the pH by using a pH meter (Ruen-ngam et al., 2018) which must be in the range between 3.5-7.5.

Assess satisfaction Prepare 2 basic serum formulas, then assess satisfaction from 36 volunteers by selecting volunteers with good skin health (according to the product industry standards TISI Certification 478-2555) by using a questionnaire for color, odor, viscosity, texture, absorption into the skin, moisture, and overall liking. From the statistical analysis from the questionnaires, we selected the better and used it for further research.

2.6 Determination of optimum conditions and stability of extracts

The preparation of the best conditions for the serums that contained the extract of *I. tenuipes* at 1.8%, 1.9% and 2.0% weight by weight, analyzed the key substances and chose the best conditions for further study.

2.7 Test for the stability of *I. tenuipes* extracts from serum

Serums, containing *I. tenuipes* extracts were stored at 4 and 30 °C, then analyzed for key substances, every three weeks, for a period of three months, by dissolving 0.2 g serum in 2 mL water. The solution was centrifuged at 5000×g for 20 minutes and the supernatant was taken for analysis.

2.8 Assessment of quality and satisfaction (physical, chemical and microbial)

Physical properties (Ruen-ngam et al., 2018). We observed creaming and cracking measured viscosity at 30°C by viscometer and color was measured in the CIE L*a*b* space with a color meter (Color Global Co., Ltd.).

Chemical properties we measured pH, using a Haida Model HD-024 Bench Top pH Meter, to check that it was in the range of 3.5-7.5 following industry standard TISI Certification 478-2555 (TISI Certification 478. 2555).

Biological properties (The Central Lab by Pathawin, 2020a, 2020b). We counted bacteria, following (USP 41, chapter 61) and Testing (USP 41, chapter 62), including *Staphylococcus aureus, Pseudomonas aeruginosa*, *Candida albicans* and *Clostridium* spp.

Assessment of stability. We stored the serum containing *I. tenuipes* extract at 4°C, 30°C (room temperature) and 45°C for 12 weeks. Color comparison by using CIE L*a*b* system, pH, viscosity (according to industrial standards).

2.9 Consumer acceptance evaluation

We prepared samples of the selected formula serum. We used 100 volunteers (Ruen-ngam et al., 2018), selected by industry standard TISI Certification 478-2555 to test the *I. tenuipes* serum, compared to commercial products. Satisfaction tests used consumer acceptance questionnaires covering color, smell, texture, absorption into the skin, moisture, and overall liking, *among other attributes.*

2.10 Statistical analysis of significantly differences

A completely randomized design and ANOVA (Minitab version 18) were used to establish relationships between variables at the 95% confidence level (p <0.05). Mean values were analyzed by Tukey's test.

3. Results and Discussion

3.1 Key substances and biological activity of *I. tenuipes* extracts

In this study, we found that *I. tenuipes* extracts contained cordycepin at 0.32 mg/g, adenosine at 5.62 mg/g, and polysaccharides at 65.8 mg/g dry mass. In comparison, a previous study using the Anthrone method for polysaccharide analysis reported 29.32 mg/g of polysaccharides (Prommaban et al., 2022), based on the method established by Southgate (1969). Interestingly, that study found no detectable cordycepin in the extract and reported adenosine at 1.95 mg/g.

However, the concentration of key bioactive compounds in *I. tenuipes* extracts can vary significantly due to several factors, including strain potency, culture medium, environmental conditions, cultivation duration, extraction methods, and analytical techniques. As such, direct comparisons between studies may be limited.

3.2 Effect of extracts from *I. tenuipes*

Antioxidant activity of *I. tenuipes* extracts was measured using DPPH and ABTS assays, with IC_{50} values of 0.27 mg/mL and 0.04 mg/mL, respectively. The inclusion of *I. tenuipe*s extract in the serum resulted in increased polysaccharide content and enhanced antioxidant activity compared to a serum without the extract. This enhancement may be attributed to the presence of polysaccharides, which have been associated with antioxidant effects (Haghparast et al., 2013). According to Fernandes and Coimbra (2023), specific structural features of polysaccharides, such as charge, molecular weight, and the presence of non-carbohydrate substituents, contribute to their antioxidant properties.

3.3 Stability of key substances from *I. tenuipes* extract

Table 2 presents the stability data (expressed as % degradation from the initial 100% at week 0) of key substances in the extract solution stored at 4°C and 30°C over a 12-week period. Polysaccharides showed less than 10% degradation under both storage conditions. However, cordycepin decreased by up to 50% at the higher temperature (Table 3). Natural extracts are generally less stable and easily decompose (Blasi & Cossignani, 2020), the extract lost physical and chemical stability, within 3 days, which was attributed to an oxidation reaction, therefore ethylenediaminetetraacetic acid (EDTA), a chelating agent, should be added, it can be concluded that other substances should be added to help stabilize the solution. As expected, storing at 4°C preserved key substances, i.e. made the extracts more stable.

Overall, storage at 4°C effectively preserved key bioactive components, confirming that lower temperatures improve extract stability. This observation aligns with previous studies reporting that extracts stored at 4°C exhibited superior physical and chemical stability, whereas those stored at elevated temperatures (e.g., 45°C) showed the highest degradation (Lourith & Kanlayavattanakul, 2013; Piljac-Zegarac & Samec, 2011).

Table 2. Temporal stability of the key substances from *I. tenuipes* extracts.

Important substance type	Polysaccharide (%)		Cordycepin (%)		Adenosine (%)	
temperature (°C)	4	30	4	30	4	30
week 0	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
week 3	96.73^{b}	95.62^{b}	89.76^{a}	72.19^{b}	100.34^{a}	95.43^{a}
week 6	95.37^{b}	95.47^{b}	72.10^{a}	57.09^{c}	96.50^{a}	93.33^{a}
week 9	93.86^{b}	93.15^{b}	80.29^{a}	57.18^{c}	97.42^{a}	94.41^{a}
week 12	94.81^{b}	93.35^{b}	77.95^{a}	55.25^{c}	100.66^{a}	93.05^{a}

Values are expressed as the mean of three replicates. Values in the same column followed by the same letter are not significantly different (p < 0.05).

3.4 Satisfaction of the basic formulae

The properties of basic serum formulas were tested (Table 4). Formula 1 had pH 6.01, viscosity of 7,547 cP and Formula 2 had a pH 4.69 and 2,946 cP, both lying in the ranges specified in the standard (TISI Certification 478. 2555). Color was measured in the CIE L*a*b* space. The basic serum Formula 1, with L* 100.43, a* -1.75, and b* -8.15, was opaque white and formula 2, with L* 13.41, a* -0.05, and b* -3.73, was clear. Neither formula showed creaming or cracking. In satisfaction testing, the volunteers preferred the Formula 2 color more than the Formula 1 color, and this difference was significant. The volunteers preferred the smell of Formula 1 and responses were also significantly different. For moisture, texture and overall liking, the volunteers preferred Formula 2, but there was no statistically significant difference. From the initial qualification test and the satisfaction tests, therefore, we chose serum formula 2 for use in the remainder of this work.

3.5 Determination of optimum conditions for *I. tenuipes* extract in serum

Levels of key substances in the various serums are shown in Table 3. Overall, cordycepin concentrations varied slightly with the *I. tenuipes* level, peaking at 2.0%, therefore 2.0% extracts were chosen, in the *I. tenuipes* serum. After that, we prepared the serum from the formula that had been selected and used it for further research.

Table 3. Key substance of *I. tenuipes* extract in serum.

Level of *I. tenuipes* extracts (%)	Key substance of *I. tenuipes* extract in serum		
	Polysaccharide mg/ml	Adenosine mg/ml	Cordycepin mg/ml
1.8	30.7[a]	1.14[a]	0.113[a]
1.9	32.8[b]	1.19[b]	0.118[ab]
2.0	35.9[c]	1.25[c]	0.123[b]

Values are expressed as the mean of three replicates. Values in the same column followed by the same letter are not significantly different ($p < 0.05$).

Table 4. Basic serum formula properties test results.

Basic serum formulas	The properties of basic serum formulas						
	pH	Viscosity (cP)	Color L*	Color a*	Color b*	Creaming	Cracking
1	6.01	7,547	100.43	-1.75	-8.15	Not detect	Not detect
2	4.69	2,946	13.41	-0.05	-3.73	Not detect	Not detect

3.6 Stability key substances in serum

The stability is shown as a percentage degradation from 100% at week 0, in Table 5. Polysaccharides, cordycepin and adenosine were reduced in comparison to those substances in the *I. tenuipes* extract, because it went through many steps in production, and was mixed with various other compounds.

Creaming is the separation of an emulsion in which the dispersed phase (typically oil or water) migrates to the top or bottom due to density differences, creating a visible layer.

Cracking is the process by which droplets merge into larger ones, eventually separating into distinct layers of water and oil, a permanent and irreversible condition.

Over the 12-week storage period, all key substances in the *I. tenuipes* serum, including polysaccharides, cordycepin, and adenosine, showed a gradual decline in concentration, with significantly greater reductions observed at 30°C compared to 4°C. (Table 5)

Polysaccharides remained relatively stable at 4°C, retaining 77.64% of the initial content by week 12. At 30°C, the retention dropped to 69.41%, with significant differences observed from week 0 as early as week 9 ($p < 0.05$).

Cordycepin exhibited the most pronounced degradation, particularly at 30°C, where only 40.87% remained at week 12, compared to 54.64% at 4°C. Statistically significant degradation began earlier at 30°C, with a noticeable drop from week 6 onward.

Adenosine also decreased more rapidly at 30°C (69.94%) than at 4°C (69.82%) by week 12. The differences were statistically significant from week 6 for both temperatures.

These results confirm that lower storage temperatures (4°C) help preserve the stability of key bioactive compounds in *I. tenuipes* serum, aligning with previous findings on temperature effects on antioxidant retention in natural extracts.

Table 5. Stability of key substances from *I. tenuipes* serum during 12 weeks of storage, sampled every 3 weeks.

Key substance	Polysaccharide (%)		Cordycepin (%)		Adenosine (%)	
Temperature (°C)	**4**	**30**	**4**	**30**	**4**	**30**
week 0	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
week 3	98.8^{a}	98.74^{a}	75.44^{ab}	75.02^{ab}	88.58^{b}	87.69^{b}
week 6	94.99^{ab}	93.30^{ab}	64.66^{ab}	55.09^{bc}	79.27^{bc}	79.94^{c}
week 9	89.75^{b}	78.58^{bc}	53.03^{ab}	35.10^{cd}	73.82^{c}	65.91^{d}
week 12	77.64^{c}	69.41^{c}	54.64^{b}	40.87^{d}	69.82^{c}	69.94^{d}

Values are expressed as the mean of three replicates. Values in the same column followed by the same letter are not significantly different ($p < 0.05$).

3.7 Physical stability of *I. tenuipes* serum

The physical stability is shown as a percentage degradation relative to 100% at 4°C in Table 6. The pH, viscosity, and color relative to those at 4°C and those at 45°C varied by less than 10%, and therefore were physically stable (considering industry standards). Therefore, for long term storage it should be kept at the right temperature (Chittasupho, 2016) because storage temperature affectes the pH, when the temperature rose, pH decreased, since the increased temperature led to increased H+ concentration in solution and thus lower pH. As expected, the viscosity also decreased with increased temperature. The color value changes due to the components that make the color in the serum being a natural extract of *I. tenuipes*. This is consistent with previous research which observed pigment changes in sweet potatoes, as temperature increased, chlorophyll which generates the color was degraded and color intensity dropped (Bravo et al., 2023; Manolopoulou & Varzakas, 2016).

Table 6. Physical stability of *I. tenuipes* serum at various temperatures for periods of 3 months.

Temperature	Physical stability of key substances in *I. tenuipes* serum				
	pH	Viscosity	Color		
			L*	a*	b*
4 °C (controlled temperature)	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
30 °C (room temperature)	96.7^{b}	98.77^{a}	96.84^{b}	101.24^{a}	96.42^{b}
45 °C	93.0^{c}	94.74^{b}	90.71^{c}	108.19^{a}	96.04^{b}

Values are expressed as the mean of three replicates. Values in the same column followed by the same letter are not significantly different ($p < 0.05$).

3.8 Microbiological analysis

We measured the numbers of bacteria, mold and yeasts and found less than 10 colonies/g. We did not identify any *S. aureus*, *P. aeruginosa*, *C. albicans* or *Clostridium* spp. *i.e.,* which conformed to industry standards.

3.9 Consumer acceptance evaluation results

A total of 100 volunteers participated in a consumer acceptance survey using a 5-point hedonic scale, comparing the *I. tenuipes* serum with a commercial product based on various sensory attributes. For color and odor, participants expressed a preference for the commercial product, with significantly higher scores than those of the *I. tenuipes* serum. However, the *I. tenuipe*s serum received significantly higher scores for moisture (4.02), texture (3.93), skin absorption (4.10), and overall liking (4.06), as shown in Figure 2. These findings suggest that while some sensory aspects of the commercial product were favored, the *I. tenuipes* serum was preferred in terms of key functional and experiential qualities.

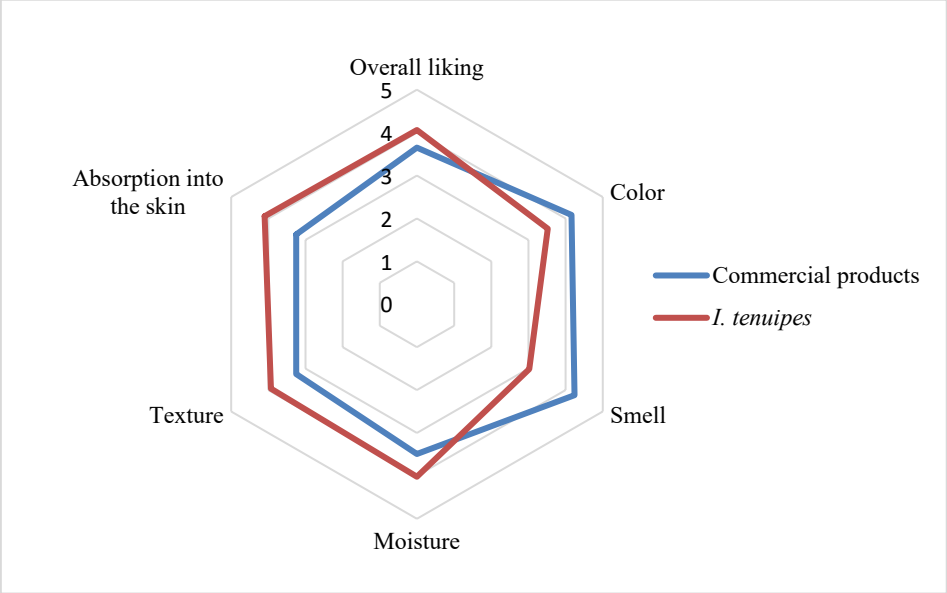

Figure 2. Spider diagram of the consumer acceptance between commercial product and *I. tenuipes* serum.

4. Conclusions

Our findings demonstrated that key bioactive substances in *I. tenuipes extract*, particularly polysaccharides and adenosine, were well preserved at storage temperatures up to 30°C, with degradation of less than 10% observed in both laboratory solutions and serum formulations. This suggests that the product can be marketed as stable at room temperature, eliminating the need for refrigeration, which is an important consideration for global distribution and extended shelf life. However, cordycepin showed greater degradation at 30°C compared to 4°C, indicating the need for further research into encapsulation technologies or formulation buffers to enhance its stability.

The serum's physical and chemical properties, including viscosity, color, and pH, remained largely unchanged throughout the storage period. Microbiological testing confirmed that microbial growth remained within acceptable industrial limits. Consumer acceptance tests revealed high preference scores for the serum containing *I. tenuipes*, particularly in terms of viscosity, texture, absorption, moisture, and overall satisfaction. However, the commercial product scored higher in terms of color and odor. This highlights the need for additional formulation research to improve sensory attributes while maintaining the stability of the active compounds. These findings support the potential of *I. tenuipes* extract for further development in both cosmetic and functional food applications.

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Conflict of Interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

ORCID

Marisa Jatupornpipat
https://orcid.org/0000-0001-5616-3659

Ethical Approval

This article did not contain any studies with human participants or animals performed by any of the authors. However, the research project has been market tested.

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